Environmental Research Laboratory. 1978. Intensive culture of Penaeid shrimp in controlled environments. Environmental Research Laboratory, University of Arizona.

Laubier-Bonichon, A., et L. Laubier. 1976. Reproduction contrôlée chez la crevette *Penaeus japonicus*. FAO Technical Conference on Aquaculture, Kyoto, Japan.

Laubier-Bonichon, A. 1978. Ecophysiologie de la reproduction chez la crevette *Penaeus japonicus*. Trois années d'expérience en milieu contrôlé. Oceanologica Acta 1-2:135-150.

Primavera, J. H. 1978. Notes on the courtship and mating behavior in *Penaeus monodon* Fabricius. Contribution No. 29, Aquaculture Department, Southeast Asian Fisheries Development Center, Box 256, Iloilo City, Philippines.

SEAFDEC. 1976. Development of a broodstock of the tiger prawn *Penaeus monodon* Fabricius. Technical Report No. 1.

Proc. World Maricul. Soc. 10:453-463 (1979)

ORGAN INDICES AND BIOCHEMICAL LEVELS OF OVA FROM PENAEID SHRIMP MAINTAINED IN CAPTIVITY VERSUS THOSE CAPTURED IN THE WILD

A. L. Lawrence,[1,2] D. Ward,[1] S. Missler,[3] A. Brown,[4] J. McVey[4] and B. S. Middleditch[3]

ABSTRACT

This paper characterizes further penaeid shrimp reproduction by reporting: (1) the gonad, hepatopancreas, tail and eyestalk indices for unablated and unilateral ablated penaeid shrimp (*P. setiferus*) maintained in captivity versus shrimp captured in the wild; and (2) the biochemical content of spawned ova from three species of penaeid shrimp (*P. setiferus*, *P. stylirostris* and *P. vannamei*) obtained from wild populations and one species (*P. setiferus*) matured and spawned in captivity. The data indicated that (1) the dietary and environmental regimen used in this study is not optimum for reproduction of *P. setiferus* in captivity; (2) the hepatopancreas but not the tail muscle is directly involved in penaeid reproduction; (3) the population of *P. setiferus* sampled on June 2 and July 22, 1978, in the northwestern Gulf of Mexico was not at its reproductive peak; (4) the percent protein, lipid and carbohydrate content of unhatched eggs of *P. setiferus*, *P. stylirostris* and *P. vannamei* are very similar; and (5) biochemical analysis of the unhatched eggs and the determination of organ indices during reproduction are important parameters to obtain for understanding and evaluating penaeid reproduction in the wild and in captivity.

[1]Department of Biology, Programs in Physiology and Marine Science, University of Houston, Houston, TX 77004.

[2]Present address: Texas Agricultural Experiment Station and Department of Wildlife and Fisheries Sciences, Texas A&M University, P.O. Box 10607, Corpus Christi, TX 78410.

[3]Department of Biophysical Sciences, University of Houston, Houston, TX 77004.

[4]Galveston Laboratory, Southeast Fisheries Center, National Marine Fisheries Service, Galveston, TX 77550.

INTRODUCTION

Shrimp mariculture on a commercial basis is at the threshold of reality. However, there is obviously need and opportunity for tremendous improvement in both the efficiency and predictability of all phases of the present procedures used for penaeid shrimp mariculture. The most limiting and critical of these phases is seed-stock availability. The best solution for the problem of seed-stock availability is an efficient and predictable system for penaeid shrimp reproduction in captivity. A procedure which will provide seed-stock by penaeid shrimp reproduction in captivity in quantities with a cost and predictability sufficient to support a commercial penaeid shrimp operation has not been reported. Various degrees of success of penaeid reproduction in captivity have been reported for several species (*Penaeus setiferus*, Johnson and Fielding, 1956; *Penaeus duorarum*, Idyll, 1971; *P. duorarum*, Caillouet, 1972; *Penaeus orientalis*, *Penaeus monodon*, Arnstein and Beard, 1975; *Penaeus aztecus*, *P. setiferus*, Duronslet et al., 1975; *Penaeus californiensis*, Moore et al., 1974; *Penaeus merguiensis*, *Penaeus japonicus*, *P. aztecus*, *Metapenaeus ensis*, Aquacop, 1975; *P. japonicus*, Laubier-Bonichon and Laubier, 1976; *P. setiferus*, *Penaeus stylirostris*, Conte, 1977; *P. stylirostris*, *P. setiferus*, Conte et al., 1977; *P. merguiensis*, *P. aztecus*, *P. japonicus*, *P. monodon*, *M. ensis*, Michel, 1977; *P. monodon*, Santiago, 1977; *P. japonicus*, Laubier-Bonichon, 1978; *P. setiferus*, Brown et al., 1979). Some aspects of the reproductive biology of penaeid shrimp have been reported for several species (*P. setiferus*, Heegaard, 1953; *P. setiferus*, Lindner and Anderson, 1956; *P. duorarum*, Eldred, 1958; *P. duorarum*, Cummings, 1961; *Metapenaeus dobsoni*, *Metapenaeus affinis*, *Penaeus indicus*, *Parapenaeopsis stylifera*, Rao, 1968; *P. setiferus*, Lindner and Cook, 1970; *M. affinis*, Pillay and Nair, 1971; *Penaeus semisulcatus*, *Metapenaeus stebbingi*, *Trachypenaeus granulosus*, Badawi, 1975). Cytological and histological characterization of penaeid shrimp maturation and gametogenesis of both laboratory matured and wild penaeid shrimp also have been reported (*P. japonicus*, Hudinaga, 1942; *P. duorarum*, Caillouet, 1972; Lu et al., 1973; *P. aztecus*, *P. setiferus*, Duronslet et al., 1975). However, weight and biochemical changes of the organs involved in reproduction and of the ova for penaeid shrimp have been reported only in one publication describing the seasonal gonad indices for *M. affinis* (Pillay and Nair, 1971) and one publication of biochemical changes in *P. setiferus* (Middleditch et al., 1979). The determination of weight and biochemical changes of organs involved in reproduction and of ova for many marine invertebrate species has greatly increased the understanding of reproduction in marine invertebrates (see reviews Giese, 1959, 1966a, 1969; Giese and Pearse, 1974; Lawrence, 1976). Thus, the purpose of this paper is to characterize further penaeid shrimp reproduction by reporting: (1) the gonad, hepatopancreas, tail muscle and eyestalk indices for unablated and unilaterally ablated penaeid shrimp (*P. setiferus*) maintained in captivity versus shrimp captured in the wild, and (2) the biochemical content of spawned ova from one species (*P. setiferus*) matured in captivity and three species (*P. setiferus*, *P. stylirostris* and *Penaeus vannamei*) obtained from wild populations.

MATERIALS AND METHODS

Animals were obtained from wild populations of *P. setiferus* from the northern Gulf of Mexico on June 2 and 12 and July 22, 1978, and of *P. stylirostris* and *P. vannamei* from the Gulf of Panama from January through June 1978. Populations of *P. setiferus* maturing and spawning in captivity were sampled on September 4, 1978. The procedure used for the maturation and spawning of these animals in captivity has been described (Brown et al., 1979).

All animals obtained from the wild were sacrificed within 18 hours of capture. The animals to be sacrificed were weighed, headed and the ovary or testes, hepatopancreas, eyestalks and eyes (only one if from a unilaterally ablated animal), and tail muscle removed, weighed and frozen immediately for biochemical analysis.

Spawned eggs (ova) from animals matured in captivity and from mature females (*P. setiferus*) from the wild were sampled within 12 hours of the time of spawning. After the number of eggs was estimated by aliquot sampling, the eggs were separated on a nylon mesh and then frozen immediately. After partial dehydration (at least 75% of the water present in the eggs is removed) of the eggs in a frostless freezer, the eggs were completely dried under vacuum in a desiccator at room temperature (22-24°C). Appropriate amounts, 3-7 mg, 3-7 mg, and 75-200 mg, of the dried eggs were used for the determination of the percent protein, carbohydrate and lipid respectively. The protein content of the eggs was determined using the colorimetric method of Lowry et al. (1951). The total carbohydrate content was estimated by boiling the dried eggs in 5% trichloroacetic acid, centrifuging, and testing the supernatant with the procedure of Dubois et al. (1956). The total lipid determination was made by extracting the lipid from the dried eggs and weighing the extracted lipid according to the method of Freeman et al. (1957). All chemicals used were reagent grade.

Data are reported in organ indices which are organ wet weights (in grams) divided by wet whole body weight (in grams) times 100 and percent protein, lipid and carbohydrate which are weight of protein, lipid and carbohydrate (in grams) respectively in the sample divided by dry weight of sample (in grams) times 100. Significance of differences was determined using the two tailed student's t test.

RESULTS

There were no significant differences in the percent protein, carbohydrate and lipid of eggs of the three species of penaeid shrimp obtained from wild populations (Table 1). The ranges of percent protein, carbohydrate and lipid for the three species were 37.3-42.3, 2.9-3.1 and 16.1-20.2 respectively. The percent protein and carbohydrate contents of ova from *P. setiferus* matured in the wild versus captivity are very similar. The percent lipid content of the ova from animals matured in captivity appears to be higher than the percent lipid level of the ova from animals matured in the wild. Further data are required to determine if this difference is significant.

Table 1. Biochemical Content of Ova from Three Penaeid Species Captured in the Wild and from One Penaeid Species Matured in Captivity*

Species	Source	Protein	Carbohydrate	Lipid
P. vannamei (6)	wild	42.0 ± 3.0^{a}	3.1 ± 0.2^{a}	16.7 ± 1.5^{a}
P. stylirostris (30)	wild	37.3 ± 1.0^{a}	3.0 ± 0.1^{a}	16.1 ± 1.8^{a}
P. setiferus (3)	wild	42.3 ± 3.5^{a}	2.9 ± 0.3^{a}	20.3 ± 2.5^{a}
P. setiferus (2)	cap.	40.1	3.1	26.5

*Values (% dry weight) represent means ±S.E.M. of the number of observations in parentheses (each sample contained 10^5 to 3×10^6 eggs as estimated by aliquot sampling). Female *P. vannamei* and *P. stylirostris* were collected from the Gulf of Panama from January through June 1978. Female *P. setiferus* was obtained from the northern Gulf of Mexico on June 2 and July 22, 1978.

[a]Protein, carbohydrate and lipid values of ova from the three species of animals from the wild are not significantly different ($p > 0.05$).

The original average body weights of the males and females used for the maturation laboratory experiment were 31 g and 35 g, respectively. After the animals were maintained in the laboratory for 74 days on the dietary regimen and environmental conditions previously described (Brown et al., 1979), the mean body weights for the unablated males and females were 35.1 and 47.7 g respectively and for the unilaterally ablated males and females, 33.9 and 44.9 g respectively (Table 2). Of the animals maintained in the laboratory, the females grew much more than the males. The mean wet body weight of each sex for the animals maintained in the laboratory at the time of their sacrifice was 7-9 g less than the mean body weight of animals of the same sex from the wild.

There are no significant differences in the mean tail muscle indices whether one compares wild to laboratory animals, male to female, or unablated to unilaterally ablated animals (Table 2).

The hepatopancreas indices were larger for the females than for the males for all three groups (i.e., wild, unablated captive and unilaterally ablated captive) with only the unilaterally ablated female hepatopancreas index not being significantly larger than unilaterally ablated male hepatopancreas index (Table 2). This lack of significance may be due to the small number of observations for the two groups. All the mean hepatopancreas indices of the males and females maintained in the laboratory were significantly larger than the respective indices of males and females from the wild except for the unilaterally ablated males versus the males from the wild. There were no significnat differences between the hepatopancreas indices of the laboratory maintained unablated males or females as compared to the unilaterally ablated males or females.

The mean eyestalk index, representing the ratio of the weight of the two eyestalks and eyes to whole body weight, of the unablated females and males is not twice the mean eyestalk index, representing the ratio of the weight of one eyestalk and eye to the whole body weight,

of the unilaterally ablated females and males respectively (Table 2). This may indicate that the remaining eyestalk and eye of the unilaterally ablated shrimp enlarges in response to the loss of one eyestalk and eye due to unilateral ablation.

Table 2. Body Weight and Hepatopancreas, Eyestalk, and Tail Muscle Indices of Shrimp (*P. setiferus*) from the Wild and from Unilaterally Ablated and Unablated Shrimp Maintained in Captivity*

Site	Eye	Sex	Obs.	Body Weight	Tail	Hepatopancreas	Eyestalk
Wild	unablated	♀	(52)	54.6 ± 1.4	37.4 ± 0.8^{c}	$2.62 \pm 0.08^{d,e}$	--
Wild	unablated	♂	(26)	43.5 ± 1.4	38.3 ± 1.2	1.89 ± 0.12^{e}	--
Captive	unablated	♀	(26)	47.7 ± 1.4	38.8 ± 1.7	3.04 ± 0.14^{d}	0.97 ± 0.02^{a}
Captive	unablated	♂	(14)	35.1 ± 1.2	39.6 ± 1.9	2.42 ± 0.11	1.13 ± 0.02^{a}
Captive	unilateral	♀	(10)	44.9 ± 2.0	38.1 ± 1.3	3.35 ± 0.42	0.70 ± 0.02^{b}
Captive	unilateral	♂	(3)	33.9 ± 1.5	41.6 ± 4.1	2.44 ± 0.53	0.78 ± 0.05^{b}

*Values represent means ±S.E.M. of the indicated number of observations in parentheses. Values for the indices and body weight are wet organ weight ÷ wet body weight x 100 and grams respectively. Half of the wild animals were collected on June 2 and half on July 22, 1978. All the animals maintained in the laboratory were collected on June 12 and sacrificed on September 23, 1978. All animals were collected from the northern Gulf of Mexico.

[a]Two eyestalks and eyes were used for the determination of these values.

[b]One eyestalk and eye was used for the determination of these values.

[c]Tail values of wild versus captive or male versus female are not significantly different ($p > 0.05$).

[d]Hepatopancreas values of females significantly greater than those for males ($p < 0.05$).

[e]Hepatopancreas values of animals from wild significantly less than unablated animals maintained in captivity ($p < 0.05$).

There are no significant differences between the mean gonad index of the females and males from the wild versus the females and males respectively which were maintained in the laboratory (Table 3). However, the high individual gonad index for a female from the wild group is considerably larger than the high individual gonad index for the unablated and unilaterally ablated females maintained in captivity. Conversely, the low individual gonad index for a female from the wild group is smaller than the low individual gonad index for the unablated and unilaterally ablated females maintained in captivity. Also, the mean gonad index for the females was significantly larger than the mean gonad index for the males for two (wild and unablated laboratory) of the three groups. Again, the lack of significance between the female and male gonad index of the unilaterally ablated group may be due to the small sample size.

Table 3. Gonad Indices of Shrimp (*P. setiferus*) from the Wild and Unilaterally Ablated and Unablated Shrimp Maintained in Captivity*

Site	Eye	Sex	Obs.	Mean ± SEM	High	Low
Wild	unablated	♀	(52)	5.07 ± 0.47[a,b]	15.00	0.53
Wild	unablated	♂	(25)	3.12 ± 0.16[c]	4.73	1.25
Captive	unablated	♀	(26)	5.20 ± 0.61[a]	10.66	1.86
Captive	unablated	♂	(14)	3.04 ± 0.22	4.00	0.27
Captive	unilateral	♀	(10)	4.48 ± 1.03	11.78	1.62
Captive	unilateral	♂	(3)	4.20 ± 1.43	5.63	2.77

*Values represent means ±S.E.M. of the indicated number of observations in parentheses. Values for the indices are wet organ weight ÷ wet body weight x 100. Half of wild animals were collected on June 2 and half on July 22, 1978. All animals maintained in the laboratory were collected on June 12 and sacrificed on September 23, 1978. All animals were collected from northern Gulf of Mexico.

[a]Female gonad indices significantly greater than male gonad indices ($p < 0.05$).

[b]Female gonad indices of all three groups are not significantly different ($p > 0.05$).

[c]Male gonad indices of all three groups are not significantly different ($p > 0.05$).

DISCUSSION

There are no reports in the literature concerning the percentage of protein, carbohydrate and lipid levels for unhatched penaeid ova. The similarity of the biochemical constituents of ova from three penaeid shrimp species suggests that the percentage of protein, carbohydrate and lipid content of the eggs of the genus *Penaeus* might be very similar. Pandian (1970, 1972) reported 43% and 49% lipid for the unhatched ova of the lobster (*Homarus gammarus*) and isopod (*Ligia oceanica*) respectively as compared to 16.79%, 16.1% and 20.2% for the unhatched ova of the penaeid species reported in this study. However, the percentages of protein and carbohydrate of unhatched ova of the lobster, isopod, and penaeid shrimp were similar.

Gonad, hepatopancreas, eyestalk and tail muscle indices were determined for animals from wild populations in order to establish a baseline which was used to evaluate the maturation of the same species (*P. setiferus*) in captivity. Gonad indices have been used to study marine invertebrate reproduction in many species (Giese and Pearse, 1974; Lawrence, 1976). Seasonal gonad indices were used to study reproduction of three decapod crustaceans (*Uca annulipes, Portunus pelagicus, Metapenaeus affinis*) (Pillay and Nair, 1971). They obtained high mean gonad indices of 11.73 and 1.11 and low mean gonad indices of 1.30 and 0.56 for females and males respectively for the penaeid shrimp (*M. affinis*). They observed a high individual gonad index of 14.09 for one female.

The mean gonad index (5.07) obtained for females from the wild in this study indicates that the *P. setiferus* sampled on June 2 and July 22, 1978, were not at their reproductive peak though obviously there was reproduction occurring in the wild as indicated by the high individual gonad indices for some wild females. In contrast, June and July have been reported as the peak spawning months for *P. setiferus* in the northern Gulf of Mexico (Lindner and Anderson, 1956; Lindner and Cook, 1970). The high individual gonad index of 15.0 for *P. setiferus* is close to the high individual gonad index for *M. affinis* (14.09). Decapod crustaceans with a definite annual reproductive cycle can have different peak reproductive times even for succeeding years (Pillay and Nair, 1971).

The dietary regimen and/or environmental conditions used in this study (Brown et al., 1979) were not optimum, even though maturation and spawning were occurring, because the mean gonad index of the females matured in captivity was less than the high mean gonad index (about 14) during the time of peak reproductive activity reported in other decapod crustaceans (Pillay and Nair, 1971). Further, the high individual gonad index for a female matured in captivity is less than the high individual gonad index for female decapod crustaceans in the wild (this study and Pillay and Nair, 1971). The seasonality of gonadal development has been associated with storage of energy in the hepatopancreas with a concomitant transfer (e.g., lipid) to the gonad during gametogenesis for asteroids, amphineurans and crustaceans (Giese, 1966a,b, 1969; Adiyodi and Adiyodi, 1974; Lawrence, 1976). Specifically, Adiyodi and Adiyodi (1971) reported that for the crab, *Parathelphusa hydrodromous*, there is a large increase in the lipid content of the ovary with a simultaneous decrease in the lipid content of the hepatopancreas at the end of the first phase of vitellogenesis (stages 2-3). Guary et al. (1974) also reported that the ovary and hepatopancreas are the main lipid storage organs and that there is an increased lipid content in the ovary during sexual development. They indicated that there may be a mobilization of lipids in the hepatopancreas in relation to vitellogenic requirements in a penaeid shrimp (*P. japonicus*). The significantly larger hepatopancreas indices for females than for males in this study also suggest an involvement of the hepatopancreas in penaeid maturation. The higher hepatopancreas indices of the female population maturing in captivity versus wild, even though the mean gonad indices are not different, suggest the possibility of a lack of mobilization of nutrient reserves from the hepatopancreas to the ovary due either to an improper diet (qualitatively and/or quantitatively) or environmental regimen. In any case, hepatopancreas indices of captive versus wild animals also suggest that the procedure used for this study (see Brown et al., 1979) is not optimum for penaeid reproduction.

Hormones play a very important role in crustacean reproduction (Adiyodi and Adiyodi, 1970, 1974). Further, eyestalk ablation is either necessary or enhances maturation and spawning in captivity for some penaeid species (Caillouet, 1972; Aquacop, 1975). A compensation in terms of increase in size of the remaining eyestalk and eye relative to body weight for the unilaterally ablated may be indicated by the eyestalk indices observed in this study. No significant differences were seen for the gonad, hepatopancreas and tail muscles indices for each sex for unilaterally ablated versus unablated animals. Data suggesting a possibly greater egg production from the unilaterally ablated females as compared to unablated females were reported by Brown et al. (1979) for *P. setiferus*.

The tail muscle indices were determined since the tail represents such a large percentage of the biomass of the penaeid shrimp. Tail muscle also served as a control organ. There is no reported mobilization of nutrient reserves from the tail muscle to ovary during penaeid maturation. The lack of significant differences between the tail muscle indices reported in this paper for any group of shrimp suggests that components of tail muscle are not directly mobilized for penaeid reproduction.

In conclusion, hepatopancreas, tail muscle and eyestalk indices and biochemistry of the eggs provided a more complete characterization and evaluation of penaeid shrimp reproduction. These data indicated participation of the hepatopancreas in penaeid reproduction and that the procedure used in this study was not optimum for penaeid shrimp reproduction.

SUMMARY

The data presented in this paper indicate that: (1) the dietary and environmental regimen used in this study is not optimum for reproduction of *P. setiferus* in captivity; (2) the hepatopancreas but not the tail is directly involved in penaeid reproduction; (3) the population of *P. setiferus* sampled on June 22 and July 23, 1978, in the northwestern Gulf of Mexico was not at its reproductive peak; (4) the percent protein, lipid and carbohydrate content of unhatched eggs of *P. setiferus*, *P. stylirostris* and *P. vannamei* are very similar; and (5) biochemical analysis of the unhatched eggs and the determination of organ indices during reproduction are important parameters to obtain for understanding and evaluating penaeid reproduction in the wild and in captivity.

ACKNOWLEDGMENTS

This work is a result of a research program sponsored in part by Texas A&M University Sea Grant College Program, supported by the National Oceanic and Atmospheric Administration, Office of Sea Grant, Department of Commerce, under Grant No. 4-7-158-44105.

LITERATURE CITED

Adiyodi, R. G., and K. G. Adiyodi. 1971. Lipid metabolism in relation to reproduction and moulting in the crab, *Paratelphusa hydrodromous* (Herbst): cholesterol and unsaturated fatty acids. Indian Journal of Experimental Biology 9:514-515.

Adiyodi, K. G., and R. G. Adiyodi. 1974. Comparative physiology of reproduction in arthropods. Advances in Comparative Physiology and Biochemistry 5:37-105.

Aquacop. 1975. Maturation and spawning in captivity of penaeid shrimp: *Penaeus merguiensis* de Man, *Penaeus japonicus* Bate, *Penaeus aztecus* Ives, *Metapenaeus ensis* de Hann, and *Penaeus semisulcatus* de Hann. Proceedings World Mariculture Society 6:123-132.

Arnstein, D. R., and T. W. Beard. 1975. Induced maturation of prawn *Penaeus orientalis* Kishinouyi in the laboratory by means of eyestalk removal. Aquaculture 6:411-412.

Badawi, H. K. 1975. On maturation and spawning in some penaeid prawns of the Arabian Gulf. Marine Biology 32:1-6.

Brown, A., J. McVey, B. S. Middleditch, and A. L. Lawrence. 1979. Maturation of the white shrimp (*Penaeus setiferus*) in captivity. Proceedings World Mariculture Society 10:(this volume).

Caillouet, C. W. 1972. Ovarian maturation induced by eyestalk ablation in pink shrimp, *Penaeus duorarum* Burkenroad. Proceedings World Mariculture Society 3:205-225.

Conte, F. 1977. Penaeid life cycle control. Pages 38-39 in J. A. Hanson and H. L. Goodwin (eds.), Shrimp and Prawn Farming in the Western Hemisphere. Dowden, Nutchinson and Ross, Inc., Stroudsburg, Pa.

Conte, F. S., M. J. Duronslet, W. J. Clark, and J. C. Parker. 1977. Maturation of *Penaeus stylirostris* (Stimpson) and *P. setiferus* (Linn.) in hypersaline water near Corpus Christi, Texas. Proceedings World Mariculture Society 8:327-334.

Cummings, W. C. 1961. Maturation and spawning of the pink shrimp, *Penaeus duorarum* Burkenroad. Transactions American Fisheries Society 90:462-468.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebera, and R. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.

Duronslet, M. M., A. I. Yudin, R. S. Wheeler, and W. H. Clark, Jr. 1975. Light and fine structural studies of natural and artificially induced egg growth of penaeid shrimp. Proceedings World Mariculture Society 6:105-122.

Eldred, B. 1958. Observations on the structural development of the genitalia and the impregnation of the pink shrimp, *Penaeus duorarum* Burkenroad. Florida State Board of Conservation, Technical Series No. 23. 26 pp.

Freeman, N. K., R. T. Lingren, Y. C. Ng, and A. V. Nicols. 1957. Serum lipide analysis by chromatography and infrared spectrophotometry. Journal of Biological Chemistry 227:449-464.

Giese, A. C. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. Annual Review of Physiology 21:547-576.

Giese, A. C. 1966a. Lipids in the economy of marine invertebrates. Physiological Review 46:224-298.

Giese, A. C. 1966b. On the biochemical constitution of some echinoderms. Pages 757-796 in R. A. Boolootian (ed.), Physiology of Echinodermata. John Wiley and Sons Inc., New York.

Giese, A. C. 1969. A new approach to the biochemical composition of the mollusc body. Oceanography Marine Biological Annual Review 7:175-229.

Giese, A. C., and J. S. Pearse. 1974. Introduction: general principles. Pages 2-49 in A. C. Giese and J. S. Pearse (eds.), Reproduction of Marine Invertebrates, Vol. I. Academic Press, Inc., New York.

Guary, J., M. Kayama, and Y. Murakami. 1974. Lipid class distribution and fatty acid composition of prawn, *Penaeus japonicus* Bate. Japanese Society of Scientific Fisheries 40:1027-1032.

Heegaard, P. E. 1953. Observations on spawning and larval history of the shrimp, *Penaeus setiferus* (L.). Publications of the Institute of Marine Science 3:73-105.

Hudinaga, M. 1942. Reproduction, development and rearing of *Penaeus japonicus* Bate. Japanese Journal of Zoology 10:305-393.

Idyll, C. P. 1971. Induced maturation of ovaries and ova in pink shrimp. Commercial Fisheries Review 33:20.

Johnson, M. C., and J. R. Fielding. 1956. Propagation of the white shrimp, *Penaeus setiferus* (Linn.) in captivity. Tulane Studies in Zoology 4:175-190.

Laubier-Bonichon, A., and L. Laubier. 1976. Reproduction control with the shrimp *Penaeus japonicus*. FAO Technical Conference on Aquacultura, Kyoto, Japan. FIR:AQ/CONF/76/E.38. 6 pp.

Laubier-Bonichon, A. 1978. Ecophysiology of reproduction in the prawn *Penaeus japonicus* three years experiment in control conditions. Oceanologica Acta 1:135-150.

Lawrence, J. M. 1976. Patterns of lipid storage in post-metamorphic marine invertebrates. American Zoologist 16:747-762.

Lindner, M. J., and W. W. Anderson. 1956. Growth, migration, spawning and size distribution of the shrimp *Penaeus setiferus*. U.S. Fish and Wildlife Service Fishery Bulletin 56:555-645.

Lindner, M. J., and H. L. Cook. 1970. Synopsis of biological data on the white shrimp, *Penaeus setiferus* (Linnaeus) 1967. FAO Fisheries Reports 57:1439-1469.

Lowry, O., N. M. Rosebrough, A. L. Farr, and R. J. Randell. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193:265-275.

Lu, C. C., W. H. Clark, and L. E. Franklin. 1973. Spermatogenesis of the decapod *Penaeus setiferus*. Journal of Cellular Biology 59:202a.

Michel, A. 1977. Penaeid life cycle control. Pages 36-38 in J. A. Hanson and H. L. Goodwin (eds.), Shrimp and Prawn Farming in the Western Hemisphere. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pa.

Middleditch, B. S., S. R. Missler, D. G. Ward, J. P. McVey, A. Brown, and A. L. Lawrence. 1979. Maturation of penaeid shrimp: dietary fatty acids. Proceedings World Mariculture Society 10:(this volume).

Moore, D. W., R. W. Sherry, and F. Montanez. 1974. Maturation of *Penaeus californiensis* in captivity. Proceedings World Mariculture Society 5:445-450.

Pandian, T. J. 1970. Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus*. Marine Biology 5:154-167.

Pandian, T. J. 1972. Egg incubation and yolk utilization in the isopod *Ligia oceanica*. Proceedings of the Indian National Science Academy, Part B, Biological Sciences 38:430-441.

Pillay, K. K., and N. B. Nair. 1971. The annual reproductive cycles of *Uca annulipes*, *Portunis pelagicus* and *Metapenaeus affinis* (Decapoda:Crustacea) from the Southwest coast of India. Marine Biology 11:152-166.

Rao, P. V. 1968. Maturation and spawning of the penaeid prawns of the southeast coast of India. FAO Fisheries Reports 57:285-302.

Santiago, A. C., Jr. 1977. Successful spawning of cultured *Penaeus monodon* after eyestalk ablation. Aquaculture 11:185-196.